PB82-119975

Principles and Procedures of Aquatic Toxicology Training Manual. Bioassays for Toxic and Hazardous Materials

Massachusetts Univ.
Amherst

Prepared for

National Training and Operational Technology Center, Cincinnati, OH

Mar 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-119975

REPORT DOCUMENTATION PAGE	1. REPORT NO. EPA 430/1-81-026	2.	3. Recipient's Accession No. PB82-119975
4. Title and Subtitle: Principles and Procedures of Aquatic Toxicology: Training Manual; Bioassays for Toxic and Hazardous Materials			5. Report Date: March 1981 6.
7. Author(s): C.Medeiros, R. Coler, and N. M. Ram			8. Performing Organization Rept. No.
9. Performing Organization Name and Address: University of Massachusetts, Department of Civil Engineering, Marston Hall, Amherst, MA 01003			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Environmental Protection Agency, National Training & Operational Technology Center, Cincinnati, Ohio 45268			13. Type of Report & Period Covered: Final 14.

15. Supplementary Notes
EPA NTOTC has been eliminated from the EPA's program due to budgetary cutbacks (effective 10/1/81).

16. Abstract (Limit: 200 words)
The manual is an introduction to the procedures which are used to evaluate the toxicity of chemical substances to aquatic life. Such toxicity data are presently used to determine permissible effluent discharge rates into the aquatic environment and in monitoring levels of contaminants in streams with respect to water quality standards. The manual discusses such topics as: general procedures and methods of toxicity testing, test conditions and protocol, calculations and reporting, interpretation of toxicity test data, and lab procedures for running screening tests, 96 hour LC 50 determinations, and toxicity threshold evaluations.

17. Document Analysis a. Descriptors
Bioassays, Toxicology, LC50

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement: National Technical Information Service	19. Security Class (This Report)	21. No. of Pages
	20. Security Class (This Page)	22. Price

(See ANSI-Z39.18) See Instructions on Reverse OPTIONAL FORM 272 (4-77) (Formerly NTIS-35) Department of Commerce

PB82-119975

United States
Environmental Protection
Agency

National Training
and Operational
Technology Center
Cincinnati, OH 45268

EPA 430/1-81-026
March 1981

EPA

Bioassays for Toxic and Hazardous Materials

Training Manual

Principles and Procedures of Aquatic Toxicology

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Principles and Procedures of Aquatic Toxicology

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Introduction

This manual will introduce you to the procedures which are used to evaluate the toxicity of chemical substances to aquatic life. Such toxicity data are presently used to determine permissible effluent discharge rates into the aquatic environment and in monitoring levels of contaminants in streams with respect to water quality standards.

The terms "bioassay," and "toxicity test" have frequently but incorrectly been used interchangeably. They, in fact, have two distinct meanings. Toxicity tests determine the concentration of a chemical which causes either death, or some altered physiological process reflecting interference with the normal life cycle of the test organism. The objective of "bioassay" on the other hand, is to adapt the biological response to determine the concentration of a specific chemical entity or constituent. It is therefore essentially the same as a chemical assay or analytical chemical measurement. Typically, bioassays are applied to determine the amount of a pesticide, metal, vitamin or antibiotic in a sample. The results of the bioassay are then converted to concentration values based on a standard curve.

Alternatively, toxicity data provide information about the mechanisms of toxicity, synergistic or antagonistic interactions with various environmental parameters and the overall impact of stresses with respect to mortality, growth, reproduction, respiration, and behavior. Consequently the data are used in determining the level of waste treatment required to meet water pollution control regulations, and in evaluating the effectiveness of waste treatment methods.

Toxicity tests have been performed by numerous investigators for well over a hundred years. Unfortunately, most of the data collected before

1951 are virtually useless due to the lack of a standardized technique that would allow comparison. In 1951, however, Doudoroff evolved such a protocol that details the procedure to be followed and emphasizes knowledge of test water quality.

General Methods and Procedures of Toxicity Testing

Brown (1973) recognized three classes of pollutant materials that may be examined by toxicity tests:

1. Materials essentially chemical ("classically" associated with poisons) metal salts, hydrogen cyanide, etc.
2. Physical materials: mineral particulates, radionuclides, hot water, hypertonic solutions, etc.
3. Biological materials: virus, bacteria, etc.

In our studies we will focus on the first class of contaminants which constitutes most of the pollution problems encountered in the aquatic environment. This is attributable to the large amount of synthetic residue introduced into the environment and the inability of most aquatic organisms to survive or continue their normal life functions in the presence of these substances.

Toxicity tests are conducted by exposing fish, invertebrates, or algal species to varying concentrations of toxicant for some period of time. Upon termination of the test the percent mortality is observed at each toxicant concentration and the LC50 (lethal concentration causing 50 percent mortality) is calculated. The percent mortality at varying exposure times may be expressed using the LC symbol. For example, a 96-hour LC50 is that concentration causing a 50% mortality in 96 hours. The use of LC followed by a percentage number also allows the designation of percentages of mortality other than 50%. For example, one can determine a LC90 to remove undesirable

species from fishing areas, or a LC10 to insure survival of fish exposed to industrial wastes. However, the LC50 is the standard measure of toxicity because the mean is a reliable measure of central tendency. LC50 is basically the same as the term LD50, (lethal dosage causing 50% mortality) which is used in pharmacology and mammalian toxicology. The converse of the LC50 term is the TLm (mean tolerance limit). This is the concentration, after some period of exposure, at which a survival of 50% is observed. An additional expression is the median effective concentration or EC50 used to designate a level that depresses some life function or process (reproduction, growth, etc.) by 50%.

Toxicity tests can be classified into 3 groups with respect to flow: static, renewal and continuous flow.

Static toxicity tests are simple and easily controlled. They are suitable for the detection and evaluation of compounds whose toxicity is associated with low oxygen demand, and high stability. The major limitations with static toxicity tests are: (1) The toxic substance being tested may be removed by volatilization, precipitation, or detoxification by the test organisms and (2) The test must be relatively short term because of the stress and, occasionally, the accumulation of metabolic by-products in a totally alien environment.

Flow-thru toxicity tests are more complicated in design, but new developments in apparatus and technique have made their usage practical. They are used mainly to test chronic toxicity of industrial effluents and chemicals that have high biochemical oxygen demands, are unstable or volatile, or are removed appreciably from solution by precipitation on the test organism. The protocol provides for well-oxygenated solutions,

non-fluctuating concentrations of the toxicant, and removal of metabolic byproducts. Furthermore, it more closely approximates the natural conditions of receiving streams, and thus permits extended exposures to detect chronic toxicity to identify "safe" concentrations of the toxicant. The shortcomings inherent in the flow-thru approach are: (1) the large amount of space needed, (2) the complicated experimental apparatus, and (3) the large amounts of wastes and diluent waters needed.

The first two of these limitations have been alleviated by the development of the simple and inexpensive serial dilution apparatus by Mount and Warner (1965). In such an apparatus incoming water is mixed with the toxicant at a specified proportion by a device called a diluter. Modern apparatus is equipped with a fail-safe which shuts off toxicant release when diluent stops or flow rate diminishes appreciably. This method assures a constant concentration of toxicant and O_2 in tested water regardless of volatility or O_2 demand.

Unlike static tests, the protocol for flow-thru testing permits feeding without danger of fouling or toxicant interaction. This allows testing organisms for their entire life cycle as well as for productive mortality-abnormality effects.

Renewal Toxicity Tests: This methodology can be considered as intermediate between static and continuous flow. It differs from the first type by having the test solution occasionally renewed. Here, organisms can be fed without accumulation of metabolites and excess food. The test concentration is brought back by replacement of the water not as gradually as the continuous flow, but by abrupt increases.

Renewal of test solution for the purpose of maintaining more or less uniform concentrations of any volatile or unstable toxic materials, and an

adequate DO can be accomplished by transferring the test animals quickly to fresh solutions. Test solutions, for such toxicants, must be renewed every 24 hours or less.

The three types of toxicity tests may be modified by the inclusion of a circulatory pump to simulate flowing water and keep the animals active.

Selection of species. Test animals should be adaptable to laboratory conditions and available in adequate numbers of a usable size. When possible, the test animals should be native to the water shed investigated, and preferably a species of recreational or commercial value. If these conditions cannot be met, the investigator may be compelled to obtain the test individuals from commercial aquatic dealers. In such instances, comparative tests should be performed to relate the sensitivity of the selected test organisms to the most sensitive endemic species.

Any unusual conditions to which the test group were exposed, e.g., pesticides or chemotherapeutic agents, should be considered and reported (Standard Methods, 1975).

Test organisms' lengths should not vary in extremes by more than 50% between the smallest and the largest. For fish, researchers however contend that a variation of more than a few millimeters is too much because the weight of a fish increases as the cube of its length. A natural population of fish, however, is varied in size, and the application of toxicity data derived from a very uniform group of fish to problems involving natural populations may be questionable. The test animals must also belong to the same age as well as size class. Immature specimens are commonly used because differences attributable to sex are minimized.

Standard Methods (1975) lists additional factors that should be considered in choosing a test organism:

1. Their sensitivity to the materials or environmental factors under consideration.

2. Their geographical distribution, abundance and availability throughout the year.

3. Availability of culture methods and knowledge of their environmental requirements.

4. Their general physical condition and freedom from parasites and diseases.

5. Also, because of the limitations of space and time, consideration must be given to the size of the organism and the length of its life cycle. Note that some studies require large organisms with long life cycles.

6. Their amenability to comparison of other toxicity data obtained in previous research or other toxicity investigations.

Preparation and acclimation of test fish. (Coler, 1979) Specimens selected for testing should be acclimated to the test temperature and the dilution water for at least ten days, but preferably, 30 days. LC50 values for pH, DO, NH_3 and a number of non-cumulative toxicants increase several fold upon long term (greater than five days) exposure to low levels or progressively increasing exposure to the specific test toxicant. Acclimation often occurs only below a certain concentration and can be transferable between similar toxicants. Effects upon chronic toxicity are virtually unknown. Imagine the consequence of stocking thousands of fish in a water body where a resident population has already acclimated to an existing level of a pollutant. Could the absence of extended acclimation contribute to the exceedingly low return rate of stocked trout in slightly polluted waters? Pre-exposure of fish to certain test condition changes is a poorly studied area. Acclimation to temperature has been investigated; however, different researchers have produced different results. The commonly used rule of thumb of "at least one day per centigrade degree of change" has no

particular scientific basis and may sometimes contradict the facts (Doudoroff, 1951). Peterson and Anderson (1969), concluded that the acclimation of metabolism to temperature change takes at least two weeks regardless of direction. Presently, it seems the longer the period the better; however, variations in other conditions associated with temperature changes in the laboratory may make this approach impractical. At any rate temperature fluctuations should not exceed 3°C/day.

Incidence of disease or death among acclimatized fish within a period of 41 days immediately preceding the test must be less than 5%.

General test conditions and protocol

Temperature. The range of temperature for test solutions must not exceed 4°C and must be appropriate; that is, 25°C for warm water fish, 15°C for cold water fish.

Dissolved oxygen content and aeration of test solutions. The DO concentration must not fall below 5 mg/L and 4 mg/L respectively for cold and warm water fish. Aeration with finely dispersed compressed air is not permissible when it will cause reduction in the toxicity of the solutions by accelerating loss of volative components.

Concentrations of toxicants used. Concentrations of dilutions of liquid industrial wastes are expressed as percent by volume, while concentrations of nonaqueous wastes and of individual chemicals are expressed as presented in terms of milligrams per liter (mg/l) or micrograms per liter (µg/l). These concentrations are conveniently expressed on a logarithmic scale.

The inclusion of any water of hydration as part of the weight of the solute, i.e., $CuSO_4 \cdot 5H_2O$, should be clearly indicated. When an impure chemical is used, all ingredients should be reported. The range of toxicant concentration to be examined by the full scale tests should include at least 2 replicates of 5 or 6 concentrations. This range must first be determined by exploratory tests.

Controls. A control, of diluent water (preferably ground or synthetic waters) without any toxicant, must be employed as a point for comparison. There should be no more than 10% mortality among the control animals during the course of any valid test with the remaining 90% appearing apparently vigorous. When an organic solvent or other dispersing agent is used to prepare test solutions, the test control should contain the maximum concentration of the solvent or dispersant to which organisms in the other solutions are exposed.

Sample size. At least 20 (10 per replicate) should be used for each experimental concentration.

Feeding. The daily feeding regimen should be interrupted 2 days before initiation of the static test. Specimens should not be fed during tests of limited duration (96 hours or less), to avoid large fluctuations in their metabolic rates, and fouling of test solutions with metabolic wastes and uneaten food. During a test of longer duration however, food is required.

Test duration and observations. The duration of all tests should be at least 48 hours (96 hour tests are preferable). When more than half

of the test fish survive at the highest test concentration, the test must be continued for a total of 96 hours.

The number of test fish in each test container must be observed and recorded at selected intervals. The number of live fish that show pronounced symptoms of intoxication and distress, such as loss of equilibrium and other markedly abnormal behavior, should also be recorded. Dead fish must be removed as soon as observed.

Physical and chemical determinations. Determinations of Ca and Mg ions, total dissolved solids (TDS) alkalinity, temperature, DO, and pH of test solutions and of measurable toxicant are normally made before introducing the fish, and again, at the end of the test. This is required also for any other constituents of the water which markedly influence the toxicity of the material tested.

Test containers. While the size and shape of the test containers has not been standardized, the depth of the test water should be uniform and at least 15 cm, and containers should be made of glass, except where more convenient materials have been shown to be nontoxic to the fish, and nonreactive with the test material (polyethylene adsorbs some organic substances which glass chelates with heavy metals). The weight of animal in a test container must not exceed 1 gram per 2 liters of solution. Sprague (1969) recommends that the aquarium volume should not exceed more than five times the hourly flow rate.

Modifications of static toxicity tests. Controlled artificial aeration of test solutions may be explored when excessive biochemical or other oxygen demand is found to interfere with the evaluation of toxicity.

However, uncontrolled or vigorous aeration is not acceptable. Pure O_2 in the form of large bubbles at a rate of 30-180 bubbles per minute is satisfactory.

Calculations and reporting.

LC50

A LC50 is a concentration which causes a 50% mortality in the experimental animals. Estimation of the LC50 by interpolation involves merely the plotting of the data on semilogarithmic coordinate paper with concentrations on the logarithmic, and percentage of survival on the arithmetic axis. The logarithmic nature of biological time and concentration (Gaddum 1953) necessitate the use of a logarithmic scale. The percent increase has more biological ilmpact than absolute increase. Since variability in fish is assumed to be distributed normally, the probit scale (based on a normal curve) may also be used for percent mortality. A straight line is drawn between two points representing survival at the two successive concentrations that were lethal to more than half, and to less than half of the fish after some period of exposure (usually equal to 2 or 4 days). The concentration at which this line crosses the 50% mortality line is the LC50.

The precision of the toxicity bioassay is limited by the normal biological variation among individuals of a species. A resolution within about 10% is sometimes attainable, but finer estimates are not to be expected.

Toxicity threshold estimates

The toxicity threshold or incipient lethal level is that level of toxicant below which 50% of the test specimens will not die from the stress

factor upon prolonged exposure. Toxicity threshold estimates are obtained by first determining the mean survival period of the test specimens at several concentrations of toxicant (Figure 1). The median survival periods are then plotted on a log scale against the log concentration of toxicant (Figure 2). The asymptote thus derived is interpreted as the toxicity threshold. The median period of survival is the time required to cause 50% lethality at a given concentration. Toxicants may produce linear or smooth curve relationships here, and the choice must be made according to the data.

Methods for determining the Incipient LC50 value (ILC50)

Several procedures have been utilized for determining ILC50 values: (1) The percent mortality at each concentration of toxicant is recorded over frequent time intervals. The median period of survival at each toxicant concentration is then estimated and is used in determining the toxicity threshold limit as shown in figure 2. (2) The percent mortality is observed several times over the entire dilution series. LC50 values after each of these periods of exposure is interpolated from the graphical procedure described previously. The ILC50 values is then evaluated from a plot of log LC50 vs. log time. (3) A more accurate estimation of the incipient lethal level, complete with confidence limits for each point may be made by selecting a time beyond that where acute toxicity ceases, and plotting percent killed (probit scale) for each concentration containing specimens that have survived to that time (Figure 3). The toxicant concentration corresponding to the 50% mortality value is identified as the incipient LC50. A good summary of references for computing confidence intervals is included in Sprague (1969, I).

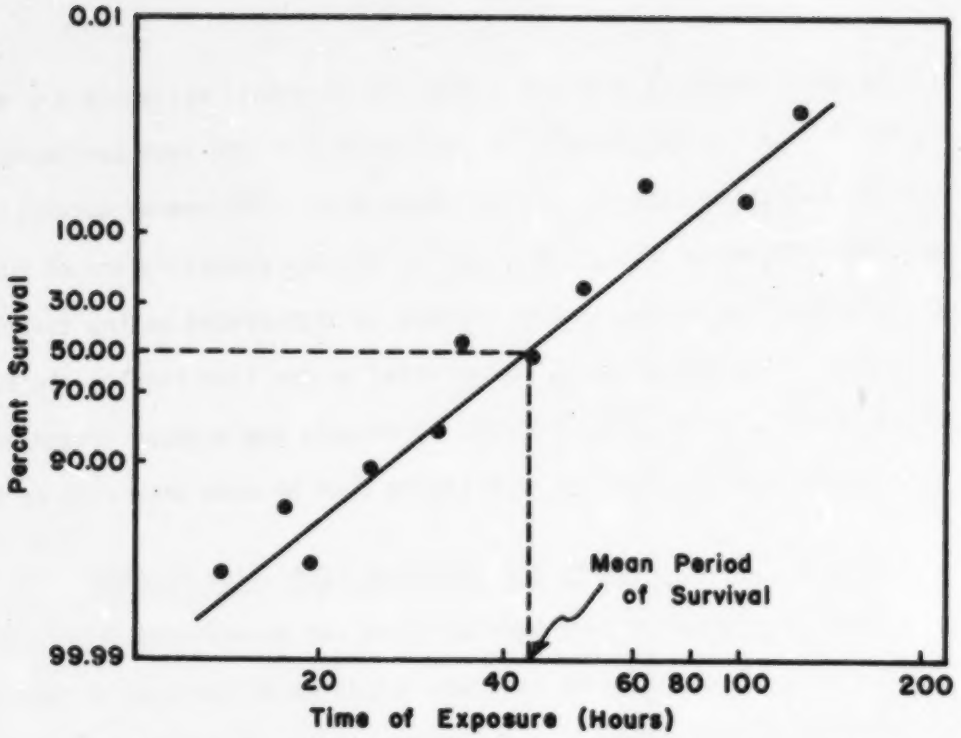

Fig. 1 Determination of Mean Period of Survival for a Given Concentration of Toxicant.

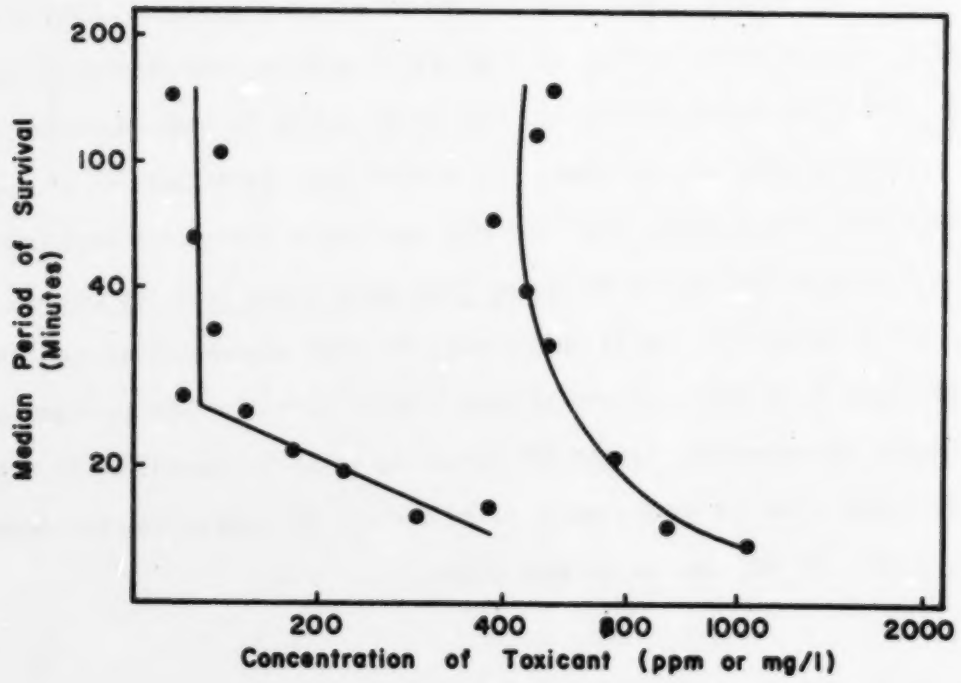

Fig. 2 Determination of Incipient Lethal Concentration.

value is identified as the incipient LC50. A good summary of references for computing confidence intervals is included in Sprague (1969, I).

Toxic units. The strength of a given toxicant may be expressed as a proportion of its threshold lethal level. Unity would represent the level corresponding to the incipient LC50. The numbers of this scale will be designated as toxic units (Sprague and Ramsey, 1965). The strength of a given toxicant may be expressed as, "toxic units -- actual concentration in solution/lethal threshold concentration." A mixture of various toxicants may be expressed as the sum of toxic units, for instance, solutions rated at five to ten toxic units might be expected to kill fish in a few hours.

This approach while a convenient simplification remains a generalization because it assumes that all effects are additive. Addition of concentrations low enough to have a minimal negative effect might result in a substantial overstatement of the resultant toxicity. Similarly, mixtures of toxicants affecting different physiological functions may produce a synergistically high value (Sprague 1969 II). While most combinations do produce additive effects, the approach is limited by a severe lack of research in the prediction, of the subtle but as profound sublethal additive effects.

Interpretation of the toxicity test. The applicability of experimental data has become an important topic in the field of fish toxicology. Numerous variables control the effects of toxicants upon fish as a whole. Such seemingly basic characteristics as temperature, dissolved oxygen, and pH are a few which can account for several-fold differences. Even more confounding is the combination of effects between a wide variety of toxicants and the physical-chemical characteristics of the total ecosystem, yet existing research has historically disregarded the assimilative and dilution

capacity of the receiving waters. Obviously, the precise evaluation of all effects is impossible. As in all real life situations, decisions must be made from fragments of information and extrapolated to large scale generalizations.

When extrapolating laboratory data to the field, one must remember that routine chronic laboratory experiments are predicated on a readily available food supply, when food is limiting in the natural environment. Also, a decrease in population or survival of fry may not result in a corresponding decrease in secondary productivity, whereas in the laboratory it most certainly will.

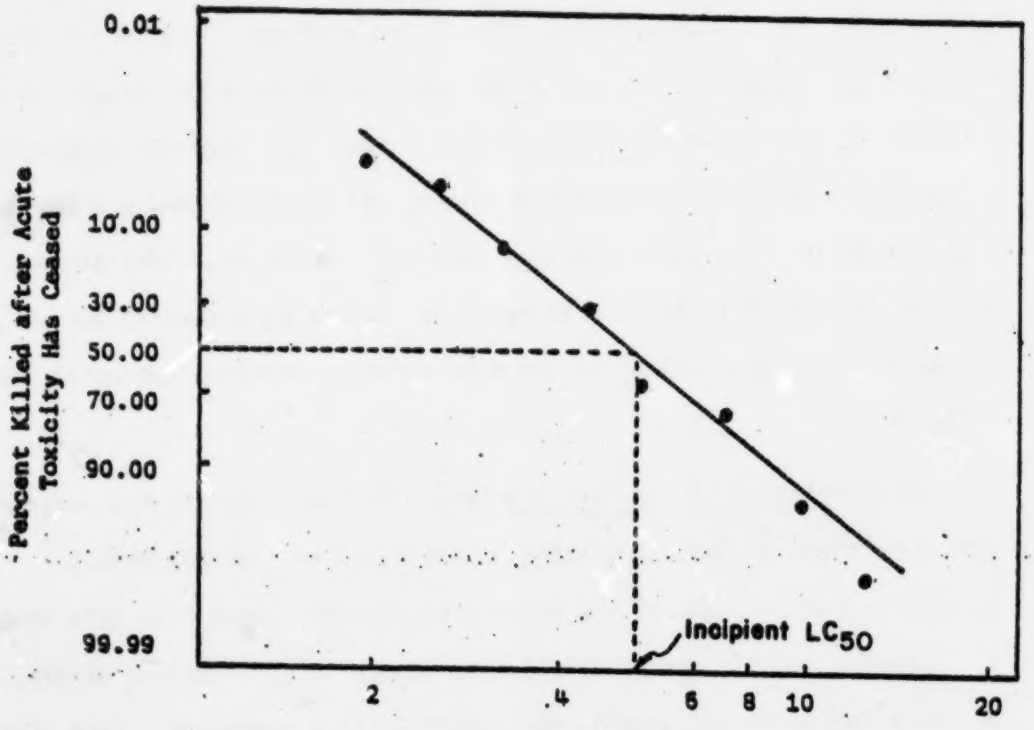

Figure 3

Biomonitoring and field verification are techniques devised, consequently, to circumvent the problem of creating artificially high or low pollution control standards in those instances where the combination of toxic effluents does not elicit an additive effect. Variable stream conditions attributable to production changes from predominately day time operations in industry, and natural diurnal fluctuation have been largely ignored. Since in natural aquatic systems organisms are rarely exposed to stable concentrations, toxicity testing should, perhaps, include some procedures for a recovery period in toxicant free water. Here again we are otherwise confronted with the possibility of creating over-protective control standards. One would expect certain combatative body functions weakened by high concentrations of certain toxicants, to be capable of replenishment during periods of low concentration. This may, in fact, necessitate varying standards for intermittent exposure based on capacity for homeostatic reserve rejuvenation.

Applications of LC values. Much work has been done on extrapolation of acceptable field concentrations of test compounds from laboratory derived LC data. However, so many variables are involved that no satisfactory "fudge factor" has yet been evolved. Somewhat of a precedent was set at one location when pulping waste was tested at a 0.1 dilution of the LC50 value and the results proved satisfactory to all concerned. Since then a basic rule of thumb used by many industries is to apply a 0.1 dilution factor to the LC50 value. Such general evaluations have justifiably received criticism from many investigators, some claiming that a 0.01 dilution of the LC50 value is a more accurate estimation of the true toxicity threshold. The relationship between the LC50 value and threshold toxicity, if one exists,

varies with the specific toxicant. One can generalize to generic types with only the greatest trepidation.

The assessment of a potential toxicant and the subsequent resolution of an application factor (AF) or its reciprocal, the chronicity value, involves the manipulation of two numbers generated by laboratory toxicity tests:

1. The 96 hour LC50 estimate of acute toxicity, or the incipient LC50 (ILC50) estimate of toxicity threshold, and
2. The safe concentration (SC) or the maximum allowable toxicant concentration (MATC).

The AF is derived by dividing the MATC by the 96 hour LC50 or the ILC50 (Standard Methods 1975). The AF, once determined, permits estimation of MATC values for a given toxicant from just the 96 hour LC50 value.

While the latter requires no great competence, estimation of the MATC is a more difficult task. The definition of some diffuse threshold of inhibition of a life function (growth fecundity, etc.) over a sustained period demands a high level of expertise. For this reason a current list of AF values would be extremely useful to laboratories evaluating sample toxicity.

Evaluation of Sample Toxicity

There are three steps required to evaluate the toxicity of a sample:

1. Determination of the range of toxicant dosage which results in an observable response.
2. Determination of the 96 LC50 or ILC50, and
3. Calculation of the MATC value.

The first step is a qualitative screening procedure used to assign a range of toxicity. One such procedure is the Ranging Oxygen Test. Several fish are placed into BOD bottles containing fully aerated dilution water (control) or dilution water plus some level of toxicant. The bottles are then sealed and left until 100 percent fish mortality is observed. The dissolved oxygen concentration in each BOD bottle is determined immediately after 100 percent mortality is obtained. The DO values in BOD bottles in which fish mortality is attributable to oxygen depletion alone will be on the order of tenths of a mg per liter. DO values in BOD bottles in which fish mortality is attributable to the level of toxicant will be higher since mortality will occur prior to oxygen depletion. Thus DO levels in BOD bottles containing higher toxicant concentrations will be greater than those containing lower toxicant concentrations. If final DO concentration is plotted against the log of the toxicant concentration, an inflection point is observed where the DO concentration increases with increasing toxicant level. Toxicant levels just below and above this point are then tested over a finer incremental dosage range for the 96 hour LC50 determination. The incremental range is distributed over a logarithmic interval owing to the exponential response of test organisms to toxicant dosage.

Alternatively, a 24 hour screening jar test can be performed over a wide range of toxicant levels. This is usually performed using ten-fold dilutions of the toxicant and observing 100 percent mortality and 100 percent survival of two test organisms at different toxicant levels after a 24 hour exposure period. The finer incremental toxicant dosage range to be used for the 96 hour LC50 toxicity test is then determined. This range occurs between the highest toxicant concentration resulting in 100 percent survival and the lowest toxicant concentration resulting in 100 percent mortality.

The second stage of evaluation is the determination of the 96 hour LC50 or ILC50. This is a static test performed with two replicates per concentration and ten fish per aquarium. The range of toxicant concentration required to observe the 96 hour LC50 value is determined using one of the qualitative screening procedures just described.

The final evalution of the toxicity of the sample is the definition of the MATC which is roughly approximated by .05 of the 96 hour LC50 or 0.1 of the 20 day LC50 (Sprague 1969 II). It is determined from the geometric mean of the greatest concentration that elicits the same response as the control and the next higher test concentration.

Lab Procedure - Screening Tests

A. Ranging Oxygen Test. Prepare solutions consisting of 100, 10, 1, .1, .01 and .001 percent of the toxicant or waste effluent using suitable dilution water. A dilution water is acceptable if healthy test organisms survive in it through the acclimation period and toxicity test without showing signs of stress, such as discoloration or unusual behavior. For effluent toxicity testing, the dilution water should be a representative sample of the receiving water (upstream water). The dilution water used in toxicity studies may, alternatively, be made from a reproducible combination of very high quality water and specified amounts of chemicals. A number of "recipes" are available in the literature for making up dilution water that are suitable to the fish and that give desired hardness and sufficient alkalinity to provide buffering capacity. The following is a simple formula providing a soft water with hardness of 40-48 mg/L as $CaCO_3$ and alkalinity of 30-35 mg/L as $CaCO_3$ (USEPA, 1978).

$NaHCO_3$	48 mg/L
$CaSO_4.2H_2O$	30 mg/L
$MgSO_4$	30 mg/L
KCl	2 mg/L

A control must be prepared as well consisting of 100 percent diluent (0 percent toxicant). Transfer test solutions into BOD bottles and then place five fish in each bottle and cap. Record the time and leave the BOD bottles undisturbed. Care should be taken when sealing the BOD bottles to avoid entrainment of air bubbles. Immediately following the death of the last test organism in each bottle record the elapsed time and determine the

DO. Note that a uniform criterion must be adopted to define the endpoint of the test (for example, cessation of gill movement).

Plot the log of toxicant concentration against DO concentrations. Note that the DO levels in the BOD bottles containing higher toxicant concentrations are greater than those containing lower toxicant concentrations in which fish mortality was observed. Determine the inflection point on the graph, and then choose a narrower range of toxicant concentration around the inflection point for more intense study.

B. Screening jar test. Prepare 3 liter solutions consisting of 100, 10, 1 .01, and .001 percent of the toxicant using suitable aerated dilution water, and pour into 1 gallon containers. A control containing no toxicant should also be used. Place 2 organisms of the same species, age, size, and sex into each container. Observe percent mortality after 24 hours. Determine the highest toxicant concentration resulting in 100 percent survival and the lowest toxicant concentration resulting in 100 percent mortality. A narrower toxicant range lying between these two values is then chosen for the 96 hour LC50 determination.

Determination of the 96 Hour LC50 and Toxicity Threshold Using the Static Jar Test

The procedure for the 96 hour LC50 determination using the static jar test is essentially the same as that described for the screening jar test. However, the test is performed over a narrower toxicant dosage range as determined from earlier screening tests. Additionally, the test is performed with 10 fish per jar and two replicates per concentration. Larger aquaria should be used to accommodate the increased number of test organisms.

Fish mortality is observed every three hours. Any additional observations such as fish turn-over, agitation, or other behavioral anomalies is also recorded.

The percentage of dead organisms (on the arithmetic scale (x axis)) vs. the concentration of toxicant (on the log scale) are plotted on semi-log paper for each of the observations taken at three hour intervals. The LC50 for each of these intervals is then determined graphically using the methods described previously.

Concurrent Testing During Time-Constrained Laboratory Exercises

Time constraints during a three day course such as the Environmental Protection Agency's Bioassay training program militate against sequentially undertaking the three phases of toxicity assessment just described. Accordingly, screening tests and the 96 hour LC50 evaluation should proceed concurrently to provide sufficient time for appropriate data collection. Thus, the raw data required for estimation of the 96 hour LC50 value will be accumulated and tabulated by the teaching staff for your use in laboratory exercises. The 5 week chronic experiment to approximate the MATC will be completed by you. However, a screening test such as the Ranging Oxygen Test is included to confirm these values and to provide adequate opportunity for course attendees to acquire the necessary laboratory skills in assessing aquatic toxicity.

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